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REVIEW

Pathogenesis of Bovine Neosporosis

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Summary

The protozoan parasite *Neospora caninum* is a major pathogen of cattle and dogs, being a significant cause of abortion in cattle in many countries. It is one of the most efficiently transmitted parasites, with up to 90% of cattle infected in some herds. The pathogenesis of abortion due to *Neospora* is complex and only partially understood. Losses occur after a primary infection during pregnancy but more commonly as the result of recrudescence of a persistent infection during pregnancy. Parasitaemia is followed by invasion of the placenta and fetus. It is suggested that abortion occurs when primary parasite-induced placental damage jeopardises fetal survival directly or causes release of maternal prostaglandins that in turn cause luteolysis and abortion. Fetal damage may also occur due to primary tissue damage caused by the multiplication of *N. caninum* in the fetus or due to insufficient oxygen/nutrition, secondary to placental damage. In addition, maternal immune expulsion of the fetus may occur associated with maternal placental inflammation and the release of maternal pro-inflammatory cytokines in the placenta. Thus *N. caninum* is a primary pathogen capable of causing abortion either through maternal placental inflammation, maternal and fetal placental necrosis, fetal damage, or a combination of all three. The question of how *N. caninum* kills the fetus exposes the complex and finely balanced biological processes that have evolved to permit bovine and other mammalian pregnancies to occur. Defining these immunological mechanisms will shed light on potential methods of control of bovine neosporosis and enrich our understanding of the continuity of mammalian and protozoal survival.

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Introduction

Neospora caninum is an intracellular protozoan closely related to *Toxoplasma gondii*. It was first described in dogs in 1984 (Bjerkås *et al.*, 1984) and later in calves with myeloencephalitis (Parish *et al.*, 1987; O'Toole and Jeffrey, 1987), but was not isolated and named until 1988 (Dubey *et al.*, 1988a, b). It may cause serious clinical illness in dogs and abortion in cattle and occasionally also in goats, sheep, deer, rhinoceros, llamas and alpacas. In horses, clinical illness has been associated with the closely related *Neospora hughesi* (Marsh *et al.*, 1995; Dubey *et al.*, 2002). *N. caninum* has been isolated from cattle (Table 1), dogs (Dubey *et al.*, 1988b, 1998b, 2004; Hay *et al.*, 1990; Cuddon *et al.*, 1992; Barber *et al.*, 1995; Marsh *et al.*, 1995; Peters *et al.*, 2000; Gondim *et al.*, 2001), sheep (Koyama *et al.*, 2001), water buffaloes (Rodrigues *et al.*, 2004), and the white-tailed deer (Gondim *et al.*, 2005; Vianna *et al.*, 2005). Antibodies to the parasite have also been reported in raccoons, camels, pigs, horses, cats, foxes, coyotes, and other wild animals (Dubey, 2003a). Primates have been infected experimentally, but evidence of *N. caninum* infection in humans is lacking. The morphology of *N. caninum* in different hosts (Dubey *et al.*, 2002) and its biology in a number of animals have been described (Dubey and Lindsay, 1996; Dubey, 2003a,b). This review focuses on the pathology and pathogenesis of neosporosis in cattle.

N. caninum: Life Cycle and Infectious Stages

N. caninum has a heteroxenous life cycle. Dogs (*Canis familiaris*) and coyotes (*Canis latrans*) are the only recognized definitive hosts for *N. caninum* (McAllister *et al.*, 1998; Gondim *et al.*, 2004b). Cattle and a wide range of other warm-blooded animals can act as intermediate hosts. There are three infectious stages of the parasite: tachyzoites, bradyzoites, and sporozoites.

Tachyzoites and bradyzoites (Fig. 1) occur in tissues of infected hosts (intermediate and definitive) whereas sporozoites are present in oocysts that are excreted in the faeces of the definitive host. Tachyzoites (Fig. 1 A–C) are lunate-shaped, measure approximately $2 \times 6\,\mu$m and have a central nucleus but lack amylopectin granules (unlike bradyzoites). They divide rapidly within cells and may infect many cell types including neural cells, vascular endothelial cells, myocytes, hepatocytes, renal cells, alveolar macrophages, and placental trophoblasts (Barr *et al.*, 1991a, b; Dubey *et al.*, 2002).

Table 1
Neospora caninum isolates from cattel

| Country	Strain	Source	Reference
Australia	NC-Nowra	Calf, 7 day old	Miller *et al.* (2002)
Italy	NC-PVI	Calf, 45 day old	Magnino *et al.* (1999,2000)
Italy	NC-PGI	Calf, 8-month old	Fioretti *et al.* (2000)
Japan	JPA-1	Calf*	Yamane *et al.* (1997)
Japan	BT-3	Adult cow	Sawada *et al.* (2000)
Korea	KBA-1	Calf, 1 day old	Kim *et al.* (1998a, 2000)
Korea	KBA-2	Fetus	Kim *et al.* (1998b, 2000)
Malaysia	Nc-MalBl	Calf*	Cheah *et al.* (2004)
New Zealand	NcNZ 1	Cow	Okeoma *et al.* (2004b)
New Zealand	NcNZ 2	Calf, 2 day old	Okeoma *et al.* (2004b)
New Zealand	NcNZ 3	Stillborn calf	Okeoma *et al.* (2004b)
Portugal	NC-Portol	Fetus	Canada *et al.* (2002)
Spain	NC-SP-1	Fetus	Canada *et al.* (2004)
Sweden	NC-SweBl	Stillborn calf	Stenlund *et al.* (1997)
UK	NC-LivBl	Stillborn calf	Davison *et al.* (1999b)
UK	NC-LivB2	Fetus	Trees and Williams (2000)
USA	BPA-1	Fetus	Conrad *et al.* (1993)
USA	BPA-2	Fetus	Conrad *et al.* (1993)
USA	BPA-3	Calf*	Barr *et al.* (1993)
USA	BPA-4	Calf*	Barr *et al.* (1993)
USA	NC-Beef	Calf*	McAllister *et al.* (1998, 2000)
USA	NC-Illinois	Calf*	Gondim *et al.* (2002)

*Clinical case.
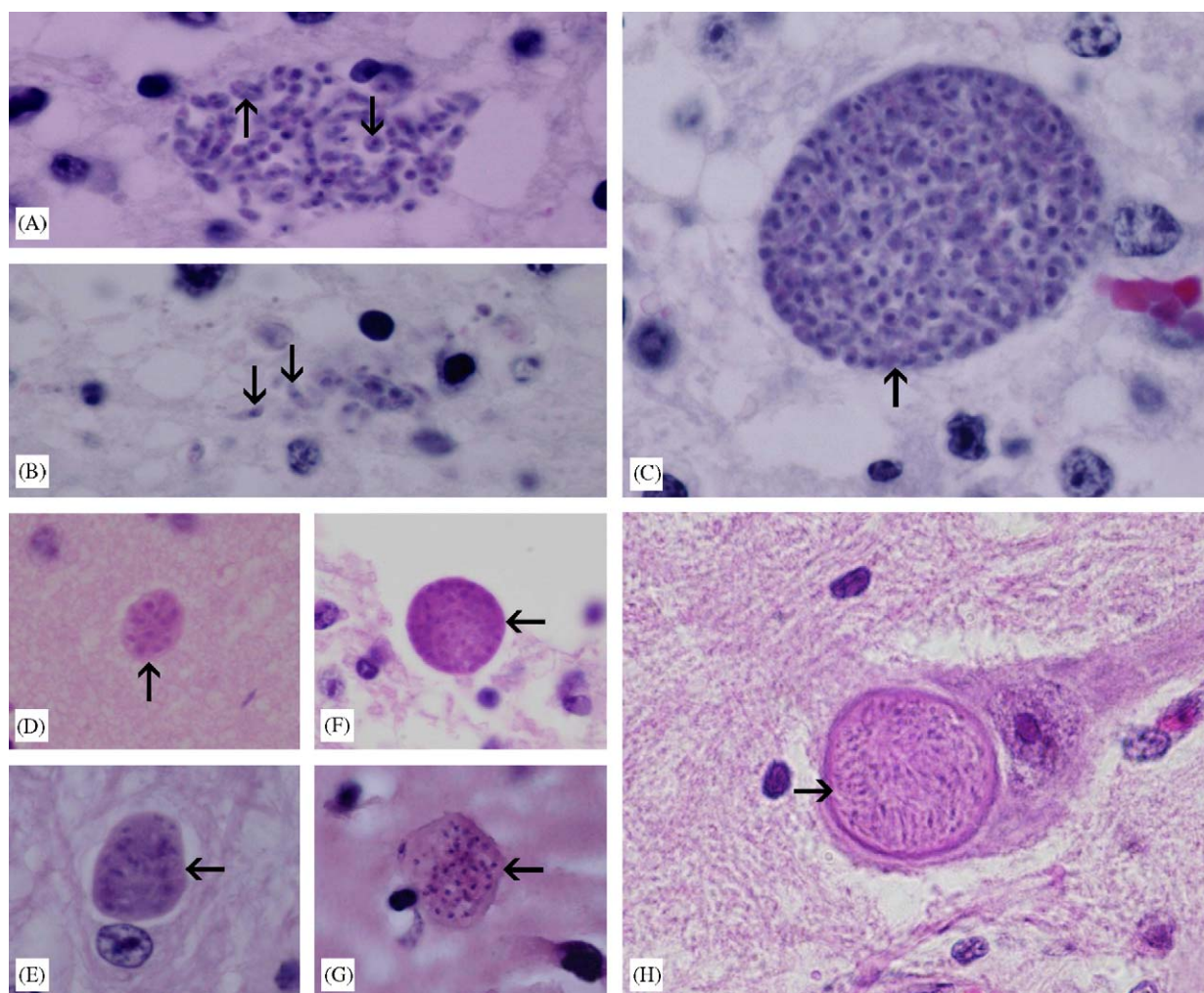
Fig. 1. A–H. *N. caninum* tachyzoites (A–C) and tissue cysts (D–H) as seen in sections of brain and spinal cord of cattle. (A) A group of tachyzoites apparently free in the brain of a fetus. Note dividing tachyzoites (arrows). (B) Extracellular crescentic forms (arrows), rarely seen in sections. (C) A large group of apparently intracellular tachyzoites (arrow). (D–G) Small tissue cysts (arrows) with varying thickness of the cyst wall in brains of aborted fetuses. (H) A thick walled (arrow) tissue cyst within a neuron in the spinal cord of a 3-day old calf. HE. ×600.

Bradyzoites are slowly replicating encysted stages of the parasite. Tissue cysts may vary considerably in size, depending on the number of bradyzoites within them (Fig. 1 D–H). In dogs tissue cysts up to 107 μm in diameter with a cyst wall up to 4 μm thick have been recorded (Dubey *et al.*, 2002). In bovine fetuses and congenitally infected calves (Fig. 1), tissue cysts are found in the brain and spinal cord and are rarely more than 50 μm in diameter with a cyst wall usually less than 2.5 μm thick (Dubey *et al.*, 1989; Barr *et al.*, 1991a, b). A few thin-walled tissue cysts have been reported in skeletal muscles of two naturally infected calves aged 2 days (Peters *et al.*, 2001). A definitive carnivore host can acquire the infection by ingestion of tissues containing tissue cysts. Bradyzoites are slender and measure approximately 6.5 × 1.5 μm (Dubey *et al.*, 2004), have a terminally located nucleus, and contain a few amylopectin granules that stain red with the periodic acid Schiff (PAS) reaction. A single tissue cyst (11 × 9 μm) was found (Table 2) in the brain of a fetus 32 days after inoculation of the dam with *N. caninum* (Dubey *et al.*, 1992b). A tissue-cyst-like structure was also described in a histopathological section of brain from a fetus 14 days after infection of the dam (Macaldowie *et al.*, 2004). In tissue cysts, however, it is difficult to identify the stage of the parasite as bradyzoites or tachyzoites in haematoxylin and eosin (HE)-stained sections, because in some cases *Neospora* can form large groups of tachyzoites while in others the thickness of the tissue cyst wall may be thin and difficult to identify (Dubey *et al.*, 2002). Bradyzoites can be definitively distinguished from tachyzoites by immunohistochemical

Table 2
Outcome of pregnancy in *Neospora*-seronegative cows inoculated with *N. caninum* during pregnancy

Number of cows inoculated	Breed	N. caninum strain (and inoculation route)	Gestational age (days)	Outcome of pregnancy	Detection of N. caninum infection in fetus		Reference
					Histology	PCR	
3	Jersey	NC-1, 2, 3 (s.c. or i.m.)	81–129	1 fetus removed 32 DAI[a] 2 aborted (74, 101 DAI)	Positive	ND	Dubey et al. (1992b)
6	Simmental	BP-1, (i.v., i.m.)	85–161	Mummified fetus 74 DAI 5 fetuses removed 26–67 DAI 1 live calf born infected (precolostral N. caninum antibody)	ND Positive Negative	ND ND ND	Barr et al. (1994)
6	Holstein-Friesian	NC-Liv (i.v.)	Up to 70	Fetuses in 5 of 6 cows died in utero at 3 weeks. The sixth cow delivered a normal uninfected calf	NR	Negative	Williams et al. (2000)
6	Holstein-Friesian	NC-Liv (i.v.)	Up to 210	All 6 cows had normal calves infected with N. caninum	NR	Positive 1/6	Andrianarivo et al. (2000)
7	Holstein	NC-BPA-1, (i.v. or i.m.)	113–122	Fetuses from all 5 cows died in utero, and aborted 26–33 DAI; all were infected	Lesions and N. caninum were detected* in 6 fetuses and in the placenta of the seventh fetus	NR	Andrianarivo et al. (2001)
5	Beef	BPA1, (i.v. or i.m.)	159–169	Fetuses removed 9 weeks after inoculation; all were live	Mild lesions in all fetuses but N. caninum detected* in only 1 fetus	NR	Trees et al. (2002)
3	Hereford-Friesian	NC-Liv oocysts (oral)	70	All 3 cows had live calves (serologically negative)	Negative	Negative	Innes et al. (2001)
6	Friesian-Holstein	NC-1 (s.c.)	140	All calves born alive, killed 6 weeks after birth	NR	Positive in 5 of 6 calves	
4	Beef-Angus	NC-Illinois (i.v.)	110	Cows killed 3–4 weeks, after inoculation	Fetuses infected, but live	Positive	Almeria et al. (2003) Maley et al. (2003); Bartley et al. (2004)
14	Holstein-Friesian	NC-1 (s.c.)	140	Cows killed at 14, 28, 42 and 56 DAI. All fetuses were live	Negative	Positive in 10 fetuses	
4	Holstein-Friesian	NC-Liv (i.v.)	70	All 4 fetuses died in utero, 3–5 weeks after inoculation	NR	Positive in 4/4 fetuses	Williams et al. (2003)
8	Holstein-Friesian	NC-1 (i.v.)	70	At 14 DAI, 2 fetuses were alive, at 28 DAI, there were no live fetuses, and at 42 and 56 DAI, no fetuses were found	Placental lesions in all cows, N. caninum not found in fetal tissues	NR	Macaldowie et al. (2004)
8	Holstein-Friesian	NC-1 (s.c.)	70	Cows were killed 14, 28, 42 and 56 DAI. At 14 DAI, there were 2 live fetuses, at 28, 42 and 56 DAI, only 3 fetuses were detected from 6 cows	Placental lesions, N. caninum not found in fetal tissues	NR	
3	Beef cows	NC-2 oocysts, (oral)	141–176	Fetal infection in 1 of 3 cows	N. caninum isolated from fetal tissues	Positive 1/3	Gondim et al. (2004a)[†]
14	Beef cows	NC-Beef oocysts (oral)	70–130	Fetal infection in 4 cows, 1 aborted calf, 1 stillborn calf, 2 healthy full-term calves	N. caninum detected by histology	Positive 4/14	
2	Beef cows	NC-IL	120	Fetal infection in 1 fetus	N. caninum isolated from fetal tissues	Positive 1/2	

DAI, days after inoculation of the dam with *N. caninum*; ND, not done; NR, not reported; s.c., subcutaneous; i.m., intramuscular; i.v., intravenous.
*Immunohistochemical labelling with anti-*N. caninum* serum.
[†]Also see text.

labelling with a bradyzoite-specific (BAG-1) antibody (McAllister *et al.*, 1996a). It is generally believed that the parasite persists as the bradyzoite stage (tissue cysts) in the tissues of adult cattle, although tissue cysts have not yet been observed in histological sections of naturally infected adult cattle. However, *N. caninum* has been isolated from the brains of two clinically normal cows that had produced infected progeny (Sawada *et al.*, 2000; Okeoma *et al.*, 2004b).

There is increasing evidence that placental tissues from naturally infected cows may be an important source of infection for dogs. *N. caninum* was consistently isolated from 20-g samples of placentas from three naturally infected cows that had delivered nine healthy but infected calves in three consecutive pregnancies (Fioretti *et al.*, 2003), and *N. caninum* oocysts were shed by dogs fed naturally infected placentas (Dijkstra *et al.*, 2001b). The former study confirms that the parasite may occur in the placenta and the latter suggests that it may be present as the bradyzoite stage. However, further research is needed to confirm this and to understand the factors that might influence the parasitic load and its viability. These factors are of considerable epidemiological significance, bearing in mind the amount of tissue (measured in kilograms) in a bovine placenta and the fact that carnivores often have easy access to placental tissues.

N. caninum oocysts, approximately 10×12 μm, are excreted in the unsporulated form in canine faeces. Sporulation then occurs so that each oocyst contains two sporocysts, each of which contains four sporozoites, individually 6.5×2 μm (Lindsay *et al.*, 1999). Experimentally, dogs have shed oocysts after ingesting naturally infected tissues from cattle (Dijkstra *et al.*, 2001b), water buffalo (Rodrigues *et al.*, 2004) and white-tailed deer (Gondim *et al.*, 2005), but to date *N. caninum* oocysts have been identified in the faeces of only a few naturally infected dogs (Basso *et al.*, 2001a; Šlapeta *et al.*, 2002; McGarry *et al.*, 2003). Currently, little is known of the frequency of shedding by canids of *N. caninum* oocysts in nature and of their viability, although dogs were shown by McGarry *et al.* (2003) to shed oocysts on more than one occasion. Seroepidemiological data also point to the importance of the dog in the life cycle of *N. caninum* (Paré *et al.*, 1998; Sawada *et al.*, 1998; Bartels *et al.*, 1999; Mainar-Jaime *et al.*, 1999; Ould-Amrouche *et al.*, 1999; Wouda *et al.*, 1999b; Basso *et al.*, 2001b; de Souza *et al.*, 2002; Dijkstra *et al.*, 2002b; Schares *et al.*, 2004; Hobson *et al.*, 2005; Rinaldi *et al.*, 2005). The schizogonic and gametogenic stages that are presumed to precede the formation of oocysts in the intestines of dogs have not yet been observed, although schizont-like stages have been reported in cell cultures seeded with bradyzoites isolated from the brains of naturally infected dogs (Dubey *et al.*, 2004)

Transmission of Infection

N. caninum is transmitted very efficiently in cattle. Both horizontal and vertical transmission routes play an important role in infection and are vital for the survival of the parasite. Horizontal transmission occurs when cattle ingest sporulated *N. caninum* oocysts. Vertical transmission is responsible for the spread of infection from a persistently infected dam to her offspring during pregnancy (Fig. 2). Postnatal transmission and congenital transmission are alternative terms used in the literature for the horizontal and vertical infection routes, respectively. Recently, the terms exogenous transplacental transmission and endogenous transplacental transmission have been proposed to describe more precisely the origin and route of infection of the fetus (Trees and Williams, 2005). Exogenous transplacental transmisson occurs after a primary, oocyst-derived, infection of a pregnant dam, while endogenous transplacental transmission occurs in a persistently infected dam after reactivation (recrudescence) of the infection during pregnancy (Fig. 2). Vertical (endogenous transplacental) transmission may lead to abortion but in the majority of cases a healthy, congenitally infected calf is born (Paré *et al.*, 1996; Anderson *et al.*, 1997; Schares *et al.*, 1998; Davison *et al.*, 1999a). Vertical transmission contributes significantly to the persistence of *N. caninum* infection in a herd by propagating the infection to successive generations (Björkman *et al.*, 1996; Anderson *et al.*, 1997; Schares *et al.*, 1998; Wouda *et al.*, 1998). Cows may remain infected with *N. caninum* for life (Trees *et al.*, 1999) and may transmit the infection to their offspring in several consecutive pregnancies (Fioretti *et al.*, 2003) or intermittently (Boulton *et al.*, 1995; Wouda *et al.*, 1998; Guy *et al.*, 2001). Detected rates of congenital infection vary, with reports of 40.7% (Pan *et al.*, 2004), 44% (Bergeron *et al.*, 2000), 63.7% (Romero and Frankena, 2003), 73% (Dijkstra *et al.*, 2003), 81% (Paré *et al.*, 1996), 85% (Björkman *et al.*, 2003), 93% (Schares *et al.*, 1998), and 95% (Davison *et al.*, 1999a). In two studies the congenital infection rate decreased with the increasing parity of the dam, suggesting that cows eventually develop a degree of immunity that prevents endogenous transplacental transmission (Romero *et al.*, 2002; Dijkstra *et al.*, 2003). In the study by Romero *et al.* (2002) of 20 Costa Rican dairy herds, the daughters born to dams with six or more parturitions had a significantly decreased probability of being seropositive as compared with daughters born to dams with one or two parturitions. In the other study, Dijkstra *et al.* (2003) analyzed 500 dam—daughter pairs in 21 Dutch dairy herds with a history of neosporosis and discovered a congenital infection rate of 80% in heifers, 71% in second parity cows, 67% in third parity cows and 66% in fourth parity and older cows (Dijkstra *et al.*, 2003).
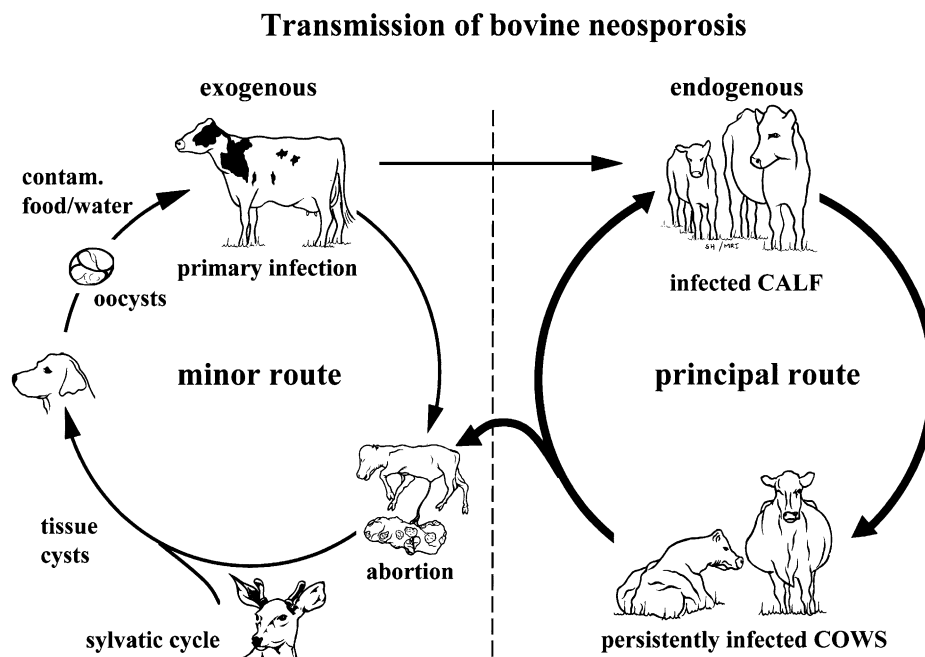
Transmission of bovine neosporosis

Fig. 2. Transmission of bovine neosporosis. Oocysts are produced by the canine definitive host and their subsequent ingestion by a susceptible pregnant cow leads to infection of the fetus (exogenous transplacental transmission). Liveborn infected heifer calves would be expected to remain infected into adulthood when they, in turn, may pass infection to their fetus (endogenous transplacental transmission). Spread of *N. caninum* in this second way is the principal route whereby the parasite is propagated in a herd.

Despite the efficiency of vertical transmission, it is evident from theoretical modelling that infection with *N. caninum* cannot be sustained in cattle herds without horizontal transmission (French *et al.*, 1999). Moreover, epidemiological studies and field observations are providing increasing evidence for the occurrence of horizontal infection in cattle. Important observations in this respect are (1) the profile of a given outbreak of *N. caninum*-associated abortions that would suggest a point-source exposure (Yaeger *et al.*, 1994; McAllister *et al.*, 1996a, 2000; Sager *et al.*, 2005), (2) an increasing sero-positivity with age (Dyer *et al.*, 2000) and (3) the lack of an association between the seropositivity of dams and daughters in infected herds (Thurmond *et al.*, 1997; Mainar-Jaime *et al.*, 1999; Waldner *et al.*, 1999; Dyer *et al.*, 2000; Dijkstra *et al.*, 2001a). The latter has been clearly demonstrated in herds facing abortion epidemics (Thurmond *et al.*, 1997; Dijkstra *et al.*, 2001a). In the Netherlands remarkable differences in seroprevalence were found between age groups in herds with *N. caninum*-associated abortions (Dijkstra *et al.*, 2001a). Animals in seropositive age groups had either seronegative dams or seronegative offspring. The first situation is indicative of horizontal infection of the daughters, and the second situation of horizontal infection of the dams, after the birth of their seronegative daughters (Dijkstra *et al.*, 2001a). Reported differences in serological response between herds with endemic and epidemic *N. caninum*-associated abortions may re-

flect the mode of infection, i.e., vertical infection in endemic cases and horizontal infection in epidemic cases (Schares *et al.*, 1999). For example, when an avidity-ELISA (Björkman *et al.*, 1999) was used to analyse a high rate of seroconversion in a dairy herd with no obvious increase of abortions, the results showed that most seropositive cows had low avidities, suggesting a recent infection of the herd (Dijkstra *et al.*, 2002a). Two other recent studies found evidence of continuous horizontal transmission of *N. caninum* in cattle herds following a point-source infection (Dijkstra *et al.*, 2002a; Björkman *et al.*, 2003). In several other studies there was a low incidence of seroconversion in endemically infected herds, suggesting a low level of horizontal infection (Paré *et al.*, 1996, 1997; Schares *et al.*, 1998; Wouda *et al.*, 1998; Davison *et al.*, 1999a; Hietala and Thurmond, 1999).

At present it appears that cow-to-cow transmission of *N. caninum* does not occur. For example, in one study 25 heifers, seronegative for *N. caninum*, were housed from birth with 25 heifers, seropositive for the parasite, and their progeny were monitored serologically for *N. caninum* infection. The seronegative heifers remained seronegative and gave birth to calves not infected with *N. caninum* while the seropositive heifers remained clinically normal but gave birth to congenitally infected calves. Seven of the congenitally infected calves (four of which were recumbent) were subjected to necropsy, and all showed histological evidence of *N. caninum*

infection (Anderson *et al.*, 1997). Theoretically, *N. caninum* may be excreted in milk or uterine discharges of infected cattle. Lactogenic transmission of tachyzoites or ingestion of fetal membranes or uterine fluids containing tachyzoites, may contribute to such infection (Schares *et al.*, 1998; Uggla *et al.*, 1998; Davison *et al.*, 2001), but it would currently seem that these routes are of little importance.

It is unlikely that *N. caninum* is transmitted venereally or by embryo transfer in cattle, and embryo transfer in particular was recommended as a method of control of endogenous transplacental transmission by Baillargeon *et al.* (2001); in this important study, infection with *N. caninum* could not be demonstrated in any of 70 fetuses or calves produced by seronegative cows that had received embryos from seropositive donors, whereas five of six calves resulting from embryos transferred from seronegative donors to seropositive recipients were infected with *N. caninum*. Landmann *et al.* (2002) confirmed these findings and showed that commercially used embryo transfer procedures also prevented transfer of *N. caninum* from seropositive cows to seronegative recipients. Furthermore, bovine embryos exposed to *N. caninum* tachyzoites before implantation appeared to be resistant to invasion by the parasite (Bielanski *et al.*, 2002). Although *N. caninum* DNA was found in the semen of naturally exposed bulls (Ortega-Mora *et al.*, 2003; Caetano-da-Silva *et al.*, 2004; Ferre *et al.*, 2005), the results suggest that viable organisms occurred infrequently and, if present, were few in number. The current view is that venereal transmission of *N. caninum* in cattle is of little importance.

Clinical Infection

Abortion is the main clinical manifestation of bovine neosporosis in both dairy and beef cattle. Fetuses dying *in utero* between 3 and 8 months of gestation are usually expelled showing moderate autolysis, but fetuses dying before five months' gestation may be mummified and retained in the uterus for several months and those dying at an early stage of gestation may be reabsorbed, with repeat breeding as a consequence (Anderson *et al.*, 1991; Barr *et al.*, 1991a; Gonzáles *et al*, 1999; Morales *et al.*, 2001; Sager *et al.*, 2001; Moore *et al.*, 2002). Abortions may be epidemic or endemic. In some herd outbreaks as many as 33% of dairy cows abort over just a few months (Thilsted and Dubey, 1989; Thornton *et al.*, 1994; Yaeger *et al.*, 1994; McAllister *et al.*, 1996b; Moen *et al.*, 1998; Wouda *et al.*, 1999a; McAllister *et al.*, 2000; Jenkins *et al.*, 2000; Dijkstra *et al.*, 2001a; Schares *et al.*, 2002). Abortions have been defined as epidemic if more than 10% or 12.5% of cows at risk abort within 6–8 weeks (Wouda *et al.*, 1999a; Schares *et al.*, 2002). A small

proportion (<5%) of cows may have repeated abortion due to neosporosis (Anderson *et al.*, 1995).

Several studies produced evidence that a recent horizontal infection preceded an outbreak of abortions (McAllister *et al.*, 2000; Dijkstra *et al.*, 2002b; Schares *et al.*, 2002) In another case, however, a horizontal infection in a dairy herd did not result in an increased incidence of abortion (Dijkstra *et al.*, 2002a). Was this herd infected with a strain of *N. caninum* of low virulence? The incidence of subclinical horizontal infections is unknown.

Seropositive dairy and beef cattle are more likely than seronegative cows to abort (Paré *et al.*, 1997; Thurmond and Hietala, 1997; Moen *et al.*, 1998; Wouda *et al.*, 1998; Davison *et al.*, 1999a; Jensen *et al.*, 1999; Atkinson *et al.*, 2000; Pereira-Bueno *et al.*, 2000; Corbellini *et al.*, 2002; Hernandez *et al.*, 2002; Pfeiffer *et al.*, 2002). However, up to 95% of calves born to seropositive dams will be congenitally infected and clinically normal (Paré *et al.*, 1996). While vertical transmission is in general more likely to occur in younger than older cows (Thurmond and Hietala, 1997; Wouda *et al.*, 1998; Dijkstra *et al.*, 2003), this is not always the case (Paré *et al.*, 1996, 1997).

Stenlund *et al.* (1999) followed fluctuations of antibodies before, during, and after parturition in 18 naturally infected heifers in a Swedish dairy herd. Of these, 13 cows were followed during the second pregnancy. Five pregnancies ended in abortion and two in stillbirth. Three of the five cows that aborted did so during the first pregnancy and two aborted during the second pregnancy. In general, antibody titres were higher in cows that aborted than in those that did not. This pattern of antibody rise was the same in both groups of cows. Although pregnancy was not synchronized, the antibody titres peaked 4–5 months before parturition and then declined from 2 months after parturition in all 18 cows, suggesting reactivation of latent infection at mid-pregnancy (Stenlund *et al.*, 1999).

Quintanilla-Gonzalo *et al.* (2000) and Pereira-Bueno *et al.* (2000) made similar observations in Spain. They studied monthly fluctuations of antibody titres during pregnancy in 32 seropositive cows, 10 of which aborted. There was an increase in antibody titres during the second trimester; this rise in titre was more prominent in cows that went on to abort than in those that calved normally.

A rise in antibody titre at 6–7.5 months of gestation reported by Paré *et al.* (1996) and by Dannatt *et al.* (1995), may have been related to a higher antigen stimulus from multiplication of the parasite in the placenta. Similar observations were made by Guy *et al.* (2001) in nine naturally infected cows, one of which aborted a *N. caninum*-infected fetus at 124 days of gestation, the fetus having been alive at 118 days of gestation. Five of the cows gave birth to live *N. caninum*-infected calves

without clinical signs, and three cows gave birth to uninfected calves. Antibodies peaked a few days before abortion in the cow that aborted, and between 155 and 250 days gestation in the five cows that gave birth to *N. caninum*-infected calves. In the three cows that gave birth to uninfected calves, there was no change in antibody values during gestation. Attempts to detect parasitaemia by polymerase chain reaction (PCR), and to detect low avidity antibodies by an avidity-ELISA, were unsuccessful (Guy *et al.*, 2001). Other than a rise in IgG antibodies, no parameter examined was indicative of congenital infection in persistently infected cows. In a more recent study of vertical transmission in naturally infected cattle, maternal parasite-specific IgG_2 antibodies rose in the second half of pregnancy (Andrianarivo *et al.*, 2005); not only did high titres of specific precolostral antibody in the live calves confirm transmission of infection but all of the live full-term calves had histopathological lesions consistent with infection with *N. caninum* infection.

Rarely, neurological signs occur in congenitally infected calves less than 1 month old. Such calves may have a below average birthweight and be unable to rise. The hind limbs or forelimbs, or both, may be flexed or hyper-extended and neurological examination may reveal ataxia, decreased patellar reflexes, and a loss of conscious proprioception. Exophthalmia or an asymmetrical appearance of the eyes has been reported and occasionally birth defects have included scoliosis, hydrocephalus and a narrowing of the spinal cord (Parish *et al.*, 1987; O'Toole and Jeffrey, 1987; Dubey *et al.*, 1990a, 1998a; Barr *et al.*, 1993; Dubey and de Lahunta, 1993; Bryan *et al.*, 1994; Peters *et al.*, 2001).

Biphasic peaks of fever, observed in closely monitored cows inoculated with *N. caninum* (Maley *et al.*, 2003; Macaldowie *et al.*, 2004) have not been recorded in naturally infected cattle. Other than fever, all experimentally infected cows (Table 2) and newborn calves (Dubey *et al.*, 1996) inoculated with large doses of *N. caninum* tachyzoites appear to remain clinically normal.

Pathogenesis

Bovine neosporosis is mainly a disease of the placenta and fetus, initiated following a maternal parasitaemia, triggered either as the result of a primary (exogenous) maternal infection or following recrudescence of a persistent (endogenous) infection during pregnancy. Because the parasite is transmitted across the placenta very efficiently and the majority of calves infected *in utero* are born healthy, it has been questioned whether the parasite is a primary cause of abortion or an opportunistic invader (Thurmond *et al.*, 1997, 1999). Evidence presented below shows that *N. caninum* is a primary

cause of abortion and that the pathogenesis of bovine neosporosis is complex.

Exogenous Transplacental Transmission

In a primary infection of a cow resulting from the ingestion of sporulated *N. caninum* oocysts (de Marez *et al.*, 1999; Trees *et al.*, 2002; Gondim *et al.*, 2004a), it is likely that the oocysts excyst in the small intestine, each releasing eight sporozoites, as in ovine toxoplasmosis (Buxton, 1998). The sporozoites then parasitise the intestinal epithelium, transform into tachyzoites and undergo a phase of multiplication, possibly in the mesenteric lymph nodes. From here, tachyzoites are released into the blood; it is not known to what extent they are intracellular or free. Okeoma *et al.* (2004a) demonstrated *N. caninum* DNA in the leucocyte fraction of blood from naturally infected cattle. The resulting parasitaemia leads to dissemination of *N. caninum* throughout the body, including the gravid uterus. Experimentally the parasitaemia is difficult to detect, presumably because it is short-lived or pulsatile, or both (Maley *et al.*, 2003; Macaldowie *et al.*, 2004).

Endogenous Transplacental Transmission

It is now firmly established that endogenous transplacental transmission of *N. caninum* is the most common mode of infection in cattle (Björkman *et al.*, 1996; Anderson *et al.*, 1997). Also seropositive cows are more likely than those that are seronegative to abort (Thurmond *et al.*, 1997; Moen *et al.*, 1998; Wouda *et al.*, 1998; Davison *et al.*, 1999a). These observations strongly suggest reactivation of an established persistent infection, possibly triggered by the "down regulation" of cell-mediated immunity that occurs around mid-gestation (Innes *et al.*, 2001, 2002, 2005). The evidence indicates that in persistently infected cattle *N. caninum* is confined to the central nervous system (CNS) and skeletal muscle (Peters *et al.*, 2001; Schares *et al.*, 2001), presumably in the form of bradyzoites within tissue cysts (Ho *et al.*, 1997; Sawada *et al.*, 2000; Okeoma *et al.*, 2004b), the parasite being in equilibrium with the dam's immune system. In human toxoplasmosis there is ample evidence to show that if host immunity is modified, a persistent infection may become reactivated and cause acute clinical illness (Wreghitt and Joynson, 2001). It is also of interest that the majority of such infections are caused by Type II *Toxoplasma gondii*, which, may induce a greater tissue cyst load than does Type I or III, and thus be more likely to cause clinical illness if host immunity is altered (Howe and Sibley, 1995). Little is known of the characteristics of *N. caninum* prevalent in infected but clinically healthy cattle, and in diseased cattle (Table 1); both genetic and biological diversity have, however, been demonstrated for some isolates

(Schock *et al.*, 2001; Okeoma *et al.*, 2004b). The data summarized in Table 2 indicate that both the canine and bovine isolates of *N. caninum* are capable of inducing disease in the bovine fetus.

Non-pregnant cattle, experimentally infected with *N. caninum*, do not develop significant clinical disease. The parasite is controlled largely by cell-mediated immune (CMI) mechanisms (Marks *et al*, 1998; Innes *et al.*, 2000; Bartley *et al.*, 2004) with cytotoxic T lymphocytes likely to have a significant protective role, demonstrable by the killing of autologous *N. caninum*-infected cells by CD4$^+$ cytotoxic T lymphocytes, a process mediated through a perforin/granzyme pathway (Staska *et al.*, 2003). This degree of protection is carried into the early stage of pregnancy. At midgestation however, immunity appears to be modified, with in-vitro tests showing a down-regulation of cellular responses to mitogen, a reduction in cell proliferation in response to specific *N. caninum* antigen, and a corresponding reduction in interferon (IFN)γ production (Innes *et al.*, 2001), suggesting that pregnancy allows reactivation of tissue cysts of *N. caninum* leading to the release of bradyzoites. These effects then gradually return to pre-pregnancy values through the rest of pregnancy. It has also been suggested that endogenous transplacental transmission may be more likely to occur in cattle that were themselves infected *in utero* (McAllister, 2001; Innes *et al.*, 2002; 2005). It is not known whether this is because they first encountered the parasite as fetuses, and as a result developed a less complete immunity to it than did cattle undergoing a primary infection when adult. As noted above, endogenous transplacental transmission rates may decrease with increasing parity, suggesting that initial immunity in the congenitally infected dam was only partly effective. There is no evidence, to date, to suggest that different types of *N. caninum* are prone to be associated with one or other mode of transmission.

How and Why does Abortion Occur?

The answer to this question is not known, but a number of hypotheses have been advanced. Following a parasitaemia, *N. caninum* is able to establish itself in the maternal caruncular septum (Maley *et al.*, unpublished data) before crossing to the fetal placental villus (Maley *et al.*, 2003; Macaldowie *et al.*, 2004). For abortion to occur, the fetus or its placenta has to be so damaged that it is no longer viable, and several factors may interact to influence this. Primary parasite-induced placental damage may (1) jeopardise fetal survival directly, or (2) cause release of maternal prostaglandins that in turn cause luteolysis and abortion (Baetz *et al.*, 1981; Foley *et al.*, 1993; Engeland *et al.*, 1996). Fetal damage may occur (1) due to primary tissue damage caused by the multi-

plication of *N. caninum* in the fetus, or (2) due to insufficient oxygen/nutrition, secondary to placental damage. In addition, maternal immune expulsion of the fetus may occur, associated with the release of maternal pro-inflammatory cytokines in the placenta. While clearly all these proposed mechanisms are related in one way or another, one or more of them may be of particular importance in a given instance and all may be influenced by the stage of gestation.

Placental Pathology and Stage of Gestation

A very young fetus will not have a sufficiently developed immune system to control parasite multiplication in the tissues, whereas a near-term fetus will be better equipped to limit parasite growth, limit the development of lesions and favour the development of tissue cysts and a persistent infection. The gestation period in cattle is *ca.* 280 days, and the fetal immune system develops progressively throughout, so that the calf is immunologically competent at birth. During the first third of pregnancy the fetus is particularly vulnerable, when the thymus, spleen and peripheral lymph nodes are first forming, but these tissues start to recognise and respond to microorganisms in the middle third of pregnancy (Osburn, 1986). For example, before 100 days gestation (dg), the bovine fetus is unable to recognise a pathogen such as bovine virus diarrhoea virus (BVDV) as being foreign (Nettleton and Entrican, 1995) and calves that survive infection at this stage are born immunotolerant to the virus, being both persistently infected with it as well as seronegative for it. Around 100–150 dg, the fetus starts to be able to mount an immune response (Osburn, 1986; Nettleton and Entrican, 1995) and after 150 dg, it becomes progressively more competent at recognizing and responding in full to various pathogens (Osburn, 1986). Thus, in the first trimester, the fetus is exceptionally vulnerable to *N. caninum* infection, and is unlikely to survive. In the middle third of pregnancy fetuses may be able to mount an immune response to *N. caninum* infection (Andrianarivo *et al.*, 2001; Almeria *et al.*, 2003; Bartley *et al.*, 2004; Innes *et al.*, 2005), which may or may not be sufficient to save it. In the third trimester the fetus is capable of an increasingly competent defence against the pathogen, leading to survival. As the majority of intrauterine transmissions result in the birth of clinically normal, infected calves, it is tempting to conclude that transmission is particularly likely to occur later in gestation (Innes *et al.*, 2005). In human toxoplasmosis, transmission from mother to fetus occurs more readily as pregnancy progresses, and this trend is inversely proportional to the fetal damage that ensues; thus, infection in early pregnancy is less likely to occur, but when it does it is lethal for the fetus (Couvreur, 2001).

The research summarized in Table 2 confirms that the fetus is most vulnerable to *N. caninum* before the 95th day of pregnancy; most of the fetuses in cows inoculated at the 70–95th day of pregnancy became infected and died, whereas those inoculated later in pregnancy were either not infected or were infected but born alive (Table 2). Of particular interest are recent results obtained by oral inoculation of pregnant cows with oocysts (Table 2). Gondim *et al.* (2004a) studied neosporosis in 19 beef cows inoculated orally with 1500 to 115 000 *N. caninum* oocysts at 70–176 days of pregnancy. Seventeen of the cows were killed for examination 65–91 days after receiving oocysts. Six cows were kept until they aborted or calved. One cow aborted an autolyzed *N. caninum*-infected fetus, 44 days after infection. Two other live fetuses were found to be infected with *N. caninum*. Two calves were healthy, but infected with *N. caninum*. One cow had a stillborn calf; the calf had mild non-suppurative encephalitis, but *N. caninum* could not be demonstrated in the tissues. Thus, at least five of the 19 cows had transplacentally infected progeny (fetuses or calves); *N. caninum* was demonstrated histologically in two (one aborted fetus and one healthy calf), *N. caninum* DNA was found by PCR in the brains of all five (and in the placenta of the aborted fetus), and the parasite was cultured *in vitro* from two (one live fetus, one healthy calf). Although data are limited, this important study indicates that the rate of transplacental infection increases with gestational age; three of four cows had infected offspring when they were fed oocysts at 162–176 days of pregnancy (Gondim *et al.*, 2004a).

Experimental infection. In six pregnant cattle inoculated in early gestation, fetal death occurred in five (Williams *et al.*, 2000). In another experiment in which eight cows were inoculated intravenously at 70 dg all eight fetuses died, while in eight cows inoculated subcutaneously fetal mortality was 50% (Macaldowie *et al.*, 2004). While the intravenous route immediately creates a parasitaemia, subcutaneous inoculation arguably more closely models a natural primary infection as the parasite is "processed" through a draining lymph node before circulating in the blood. Cows inoculated intravenously later in gestation gave birth to live calves, all with evidence of congenital *N. caninum* infection (Williams *et al.*, 2000). Similarly in cattle inoculated subcutaneously at 140 dg (Maley et al., 2003) lesion were shown to develop and then regress. In a parallel experiment (Innes et al., 2001), that used the same inoculum, cattle were inoculated by the same route at the same stage of pregnancy, and allowed to proceed to calving. These cows produced live calves that were congenitally infected with *N. caninum*. In these two studies the subcutaneous inoculation of NC1 tachyzoites led to infection of the fetus but not to fetal death. The transitory development of placental lesions (Maley *et al.*, 2003) therefore represents the mode whereby transmission of infection from mother to fetus occurred, with maternal and fetal control of parasite multiplication, placental necrosis that did not trigger abortion and maternal and fetal inflammation that resolved without precipitating fetal death (Innes *et al.*, 2005).

Placental Pathology and the Risk to the Fetus

In mammals, complex immunological mechanisms have evolved to allow the dam to nurture a fetus that is genetically a "foreign body" (allograft) rather than to reject it (Entrican, 2002). In cattle, and other ruminants, the placentation is cotyledonary and consists of up to 100 placentomes. Each placentome is composed of a fetal placental cotyledon made up of a mass of villi that sit within, and intimately interdigitate with, the honeycomb structure of the maternal caruncle that projects from the inner surface of the uterus (Noden and de Lahunta, 1985). The placenta is a dynamic tissue that constantly grows and remodels itself throughout gestation, with its initial, fairly simple villous/septal structure developing into a highly complex unit with secondary and tertiary branching of villi that sit within equally complex septal crypts. Nutrients and oxygen are transferred from mother to fetus across this interface where local maternal immunity is modulated to permit the dam to accommodate and nurture the developing "foreign" fetus. Furthermore, the placenta plays an important role in the endocrinological control of pregnancy by the production of progesterone and the metabolism of prostaglandins (Reimers *et al.*, 1985; Thatcher *et al.*, 2001; Kindahl *et al.*, 2002).

A very precise maternal and fetal immunological balance pertains in the placenta. Central to this process are cytokines, soluble mediators secreted locally that allow the producing cell to exert a powerful local effect on certain other cells of lymphoid and non-lymphoid origin (Entrican, 2002). During pregnancy, maternal immune responses in the placenta are modified to favour a micro-environment dominated by "beneficial" cytokines such as the haemopoietic cytokines (colony stimulating factor-1 [CSF-1] and granulocyte-macrophage CSF [GM-CSF]), the regulatory cytokines (transforming growth factor beta [TGF-β] and interleukin-10 [IL-10]) and the Th2-type cytokines (interleukins 4 and 5) (Entrican, 2002). Intracellular pathogens, such as *N. caninum*, stimulate cell-mediated immune responses which, in turn, invoke cytokines that may be harmful to pregnancy such as the Th1-type (inflammatory) cytokines, interferon gamma (IFN-γ) and interleukin-2 (IL-2) and the proinflammatory cytokine tumour necrosis factor-alpha (TNF-α) (Entrican, 2002). These, if present at all in the placenta, are

at low concentrations, but if the stimulus from *N. caninum* infection is sufficient it is suggested that their production will not be adequately suppressed by the beneficial cytokines, the balance will tip in their favour, and they will terminate pregnancy and trigger abortion (Innes *et al.*, 2002; Quinn *et al.*, 2002). In some instances, therefore, relatively small numbers of the parasite, causing relatively little local damage, might cause a very considerable effect by locally eliciting cytokines that endanger pregnancy. Thus while fatal for the fetus, this benefits the mother, allowing her to survive to breed again (Innes *et al.*, 2005); indeed, *N. caninum* does not affect the fertility of high-producing dairy cows (Lopez-Gatius *et al.*, 2005).

Similarly it has been suggested that placental infection and inflammation may trigger prostaglandin-induced luteolysis, causing premature uterine contraction and fetal expulsion (Baetz *et al.*, 1981; Foley *et al.*, 1993; Engeland *et al.*, 1996). To what extent the immune expulsion described and prostaglandin-induced luteolysis overlap or interact is not known; moreover, whether the former will have more influence on infections earlier in gestation and the latter be more influential later in gestation, is not known. Insufficient oxygen supply due to placental insufficiency during late gestation may trigger a fetal adrenocorticotrophic hormone (ACTH) release and subsequent premature fetal adrenal stimulation. Increased concentrations of fetal cortisol may induce oestrogen and prostaglandin-F2α secretion by the placenta, resulting in luteal regression and decreasing placental progesterone secretion. This mechanism may be responsible for late abortion or premature birth of *N. caninum*-infected calves (M.A.M. Taverne, pers. comm.).

Placental Pathology

In experimental infections the most severe lesions are normally found in the placenta (and brain of the fetus) (Table 2). Experiments have shown that when *N. caninum* invades cells in the bovine uterus, it causes focal destruction by multiplying in both maternal (Maley *et al.*, unpublished data) and fetal tissue at the materno-fetal interface and elicits a largely non-suppurative inflammatory response (Maley *et al.*, 2003; Macaldowie *et al.*, 2004). The earliest lesions in cows inoculated with tachyzoites at 70 dg gestation were seen 14 days later (Macaldowie *et al.*, 2004). They consisted of parasite multiplication in fetal placental villi (Fig. 3A) with villous necrosis, sometimes with serum leakage between
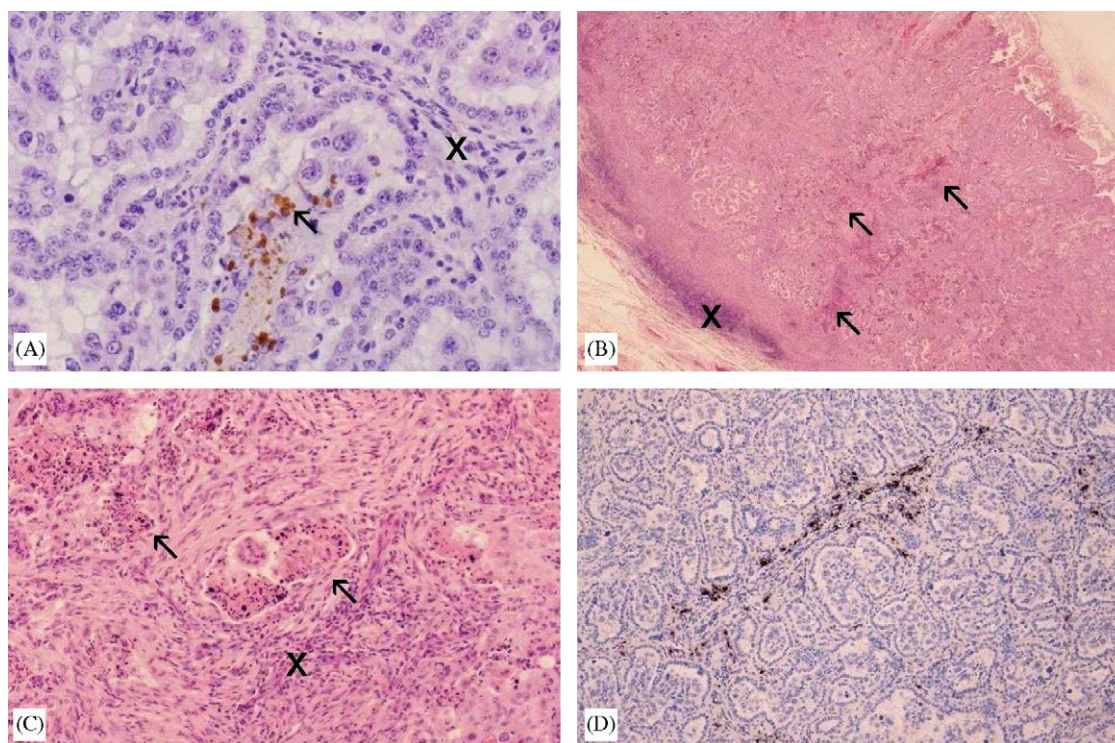

Fig. 3. A–D Histological sections of placentomes from cows 14 days after intravenous (A,D) or 28 days after subcutaneous (B,C) inoculation with NC1 *N. caninum* tachyzoites. (A) Note the inflammation in the maternal caruncular septum (×) and numerous *N. caninum* tachyzoites in a fetal placental villus (arrow). Immunohistochemistry. × 110 (B) Note the maternal inflammation at the base of the caruncle (×) and maternal septal inflammation and fetal villous necrosis (arrows). HE. × 10. (C) Inflammation in the maternal caruncular septum (×) and fetal villus necrosis (arrows). HE. × 50. (D) Inflammation in the maternal caruncular septum, with many cells labelled positively for mRNA for interferon gamma (arrows). In situ hybridization. × 50.

fetal villus and maternal septum, and non-suppurative inflammation in the maternal septa (Fig. 3C and D). Preliminary analysis has shown that the influx of maternal inflammatory cells was composed in large part of CD4+, CD8+ and γδ T-cells, and in-situ hybridization showed a proportion of the cells in the infiltrate to be capable of producing IFN-γ (Fig. 3D) (Innes *et al.*, 2005; Maley *et al.*, unpublished data). This lends support to the intriguing suggestion above that, in some cases, fetal death is less a direct result of parasite replication and more due to the maternal immune response, triggered by the parasite (Innes *et al.*, 2005). At this early stage of gestation fetal inflammation was largely absent. In cows inoculated intravenously at 70 dg all fetuses were lost after 14 days. In cows inoculated subcutaneously lesions were seen in only half the cows infected. At 28 days after injection there was breakdown of the placentome with separation of fetal cotyledons from maternal caruncles (Macaldowie *et al.*, 2004). At later timepoints, autolysis of the maternal caruncular tissues and the fetal elements of the placenta was found to have been rapid and maternal uterine tissues were returning to normal, with re-epithelization of the resolved caruncles. Descriptions of the natural disease in later infections record that a non-suppurative placentitis may also extend out into the intercotyledonary chorioallantois (Otter *et al.*, 1995) and, with time, varying degrees of mineralization of the villous connective tissue may take place (Shivaprasad *et al.*, 1989; Barr *et al.*, 1990, 1991a; Otter *et al*, 1995; Bergeron *et al.*, 2001).

Fetal Pathology

Coincidental with the onset of placental infection, the parasite enters the fetal bloodstream and invades further tissues, with a predilection for the CNS (Macaldowie *et al.*, 2004). Here, *N. caninum* is initially located in and around blood vessels (Barr *et al.*, 1991a; Dubey *et al.*, 1992b) and, in the younger fetus, its uncontrolled multiplication can cause lethal widespread destruction of the neuropil, with little or no inflammation (Fig. 4A,B) (Ogino *et al.*, 1992; Buxton *et al.*, 2002; W. Wouda, unpublished data). In older fetuses, better able to respond to the parasite, multiplication is more restricted, and necrosis is confined to small foci surrounded by a relatively intense fetal inflammatory infiltrate containing microglia, reactive astrocytes and cells of the monocyte and lymphoid series (Fig. 5A to D) (Barr *et al.*, 1994; Otter *et al.*, 1995; Wouda *et al.*, 1997; Schock, *et al.*, 2000); these foci may become mineralized (Boulton *et al.*, 1995; Gonzáles *et al.*, 1999). Associated mild meningitis may also be present. Aborted fetuses infected with *N. caninum* have multifocal necrosis and widespread mononuclear infiltrations in many tissues. Destruction of fetal cells and associated lym-
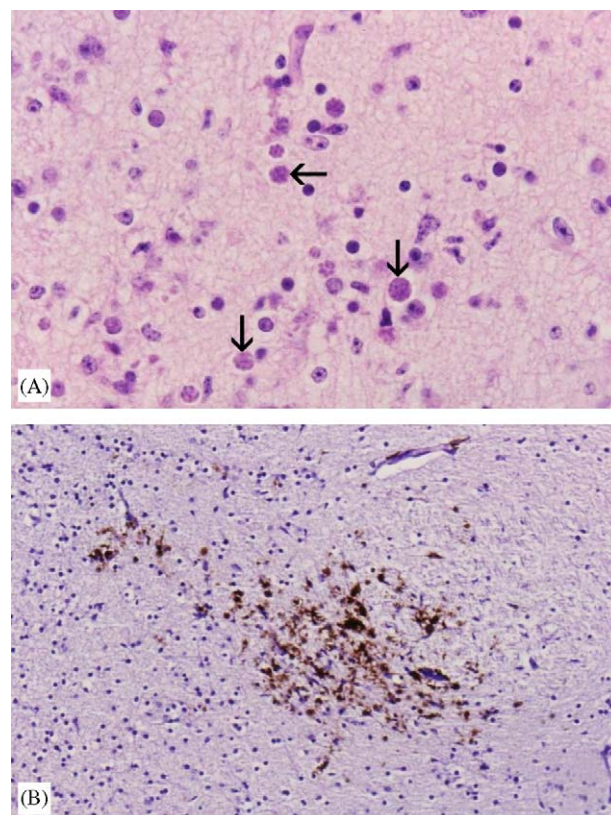

Fig. 4. A,B. Histological section of fetal bovine brain (at 84 days gestation) 14 days after inoculation of the dam with NC1 *N. caninum* tachyzoites. (A) Clusters of *N. caninum* (arrows) in the pons. HE. × 360. (B) *N. caninum* antigen in a focus of acute necrosis in the midbrain. Immunohistochemistry. × 120

phoid inflammation may occur in several tissues including the heart (Fig. 6), skeletal muscle, lung and liver (Anderson *et al.*, 1991; Barr *et al.*, 1991a; Wouda *et al.*, 1997). In some fetuses *N. caninum* may cause characteristic lesions of inflammation and necrosis, with demonstrable parasites, in tissues such as the liver and heart, while in the brain focal leucomalacia, indicative of fetal hypoxia just before birth, may be seen (S. Scholes per. comm.). Thus, *N. caninum* is a primary pathogen capable of causing abortion through maternal placental inflammation, maternal and fetal placental necrosis or fetal damage, or a combination of all three (Table 2).

Further Discussion of the Pathology of Natural Infections

Grossly, infected fetuses may often be autolysed or mummified, or both, but other macroscopic changes, although rare, have been recorded in the heart, skeletal muscle, and brain. In a fetus that was aborted at 7 months of gestation (Dubey *et al.*, 1998a) there was hydrocephalus, associated with dilated lateral ventricles and hypoplasia of the cerebellum and medulla. In a further case the heart of a full-term stillborn calf was
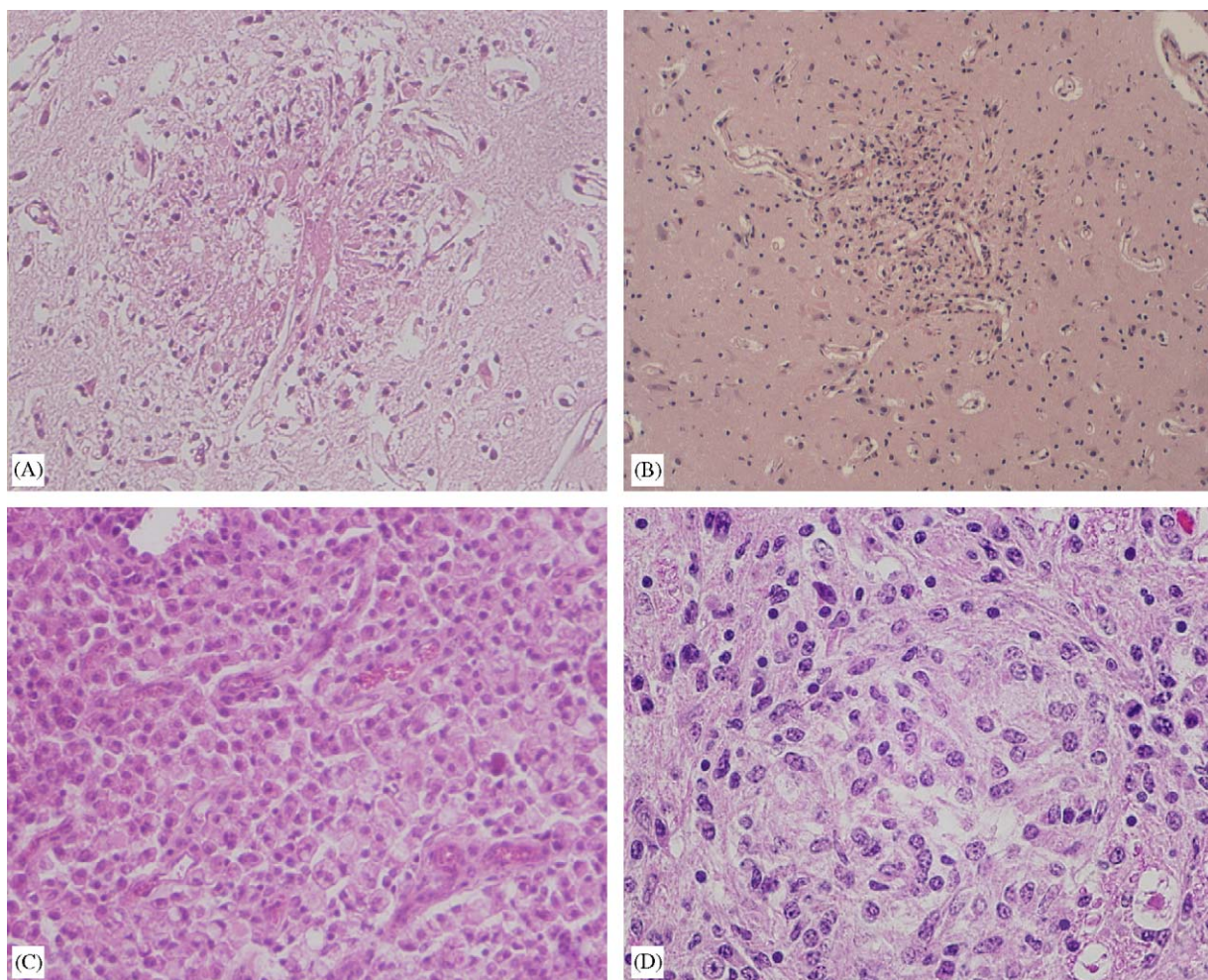
Fig. 5. A–D. Encephalomyelitis associated with *N. caninum* in naturally infected fetuses and calves. (A) Focal central necrosis with scattering of mononuclear cells at the periphery. HE. × 150. (B) Focal encephalitis. HE. × 250. (C) A 6 mm area of necrosis now occupied by macrophages in the brain of a 4-week old calf. HE. × 300. (D) Focal inflammation, largely glial, in the spinal cord of a 3-day old calf. HE. × 450.

enlarged (Dubey *et al.*, 1990b); in yet another, pale white foci were noted in cardiac and skeletal muscle, and minute pale to dark foci of necrosis in the brain (Fioretti *et al.*, 2003). The latter authors describe focal areas of discolouration in placental cotyledons. Microscopic lesions in infected fetuses are degenerative or inflammatory, or both, and may be found in a number of tissues, but are most common in the CNS (Fig. 5), heart (Fig. 6), skeletal muscle and liver, as well as the placenta (Fig. 7) (Barr *et al.*, 1990, 1991a; Anderson *et al.*, 1991; Nietfeld *et al.*, 1992; Wouda *et al.* 1997; Dubey *et al.*, 1998a; Hattel *et al.*, 1998; Helman *et al.*, 1998; Morales *et al.*, 2001; Boger and Hattel, 2003).

In a study of 82 fetuses in California, encephalitis and myocarditis were seen in 100%, adrenalitis in 80%, myositis in 72%, nephritis in 66%, hepatitis in 62%, placentitis in 53% and pneumonia in 44%. The inflammatory infiltrate was mononuclear and protozoa were observed in 89% of these fetuses (Barr *et al.*, 1990,

1991a). In a Dutch study of 80 aborted bovine fetuses with confirmed neosporosis, the brain, heart and liver were compared in respect of histopathological lesions and the distribution of *N. caninum*. Histopathological lesions were seen in all three tissues in 73 cases (91%). In the remaining seven cases, lesions were seen in two of the three tissues (Wouda *et al.*, 1997). While any part of the CNS may be affected, in a study limited to six fetuses lesions were found to occur more frequently in the cerebral grey matter than in the medulla and cerebellum (Helman *et al.*, 1998).

Encephalomyelitis was the predominant lesion in calves born live but with overt or incipient clinical illness (Parish *et al.*, 1987; O'Toole and Jeffrey, 1987). In a group of 20 such calves that were subjected to necropsy by 2 weeks of age, encephalomyelitis was the predominant finding (Barr *et al.*, 1991b, 1993; Dubey and Lindsay, 1996; Anderson *et al.*, 1997; Peters *et al.*, 2001). Lesions were more obvious in the spinal cord than in
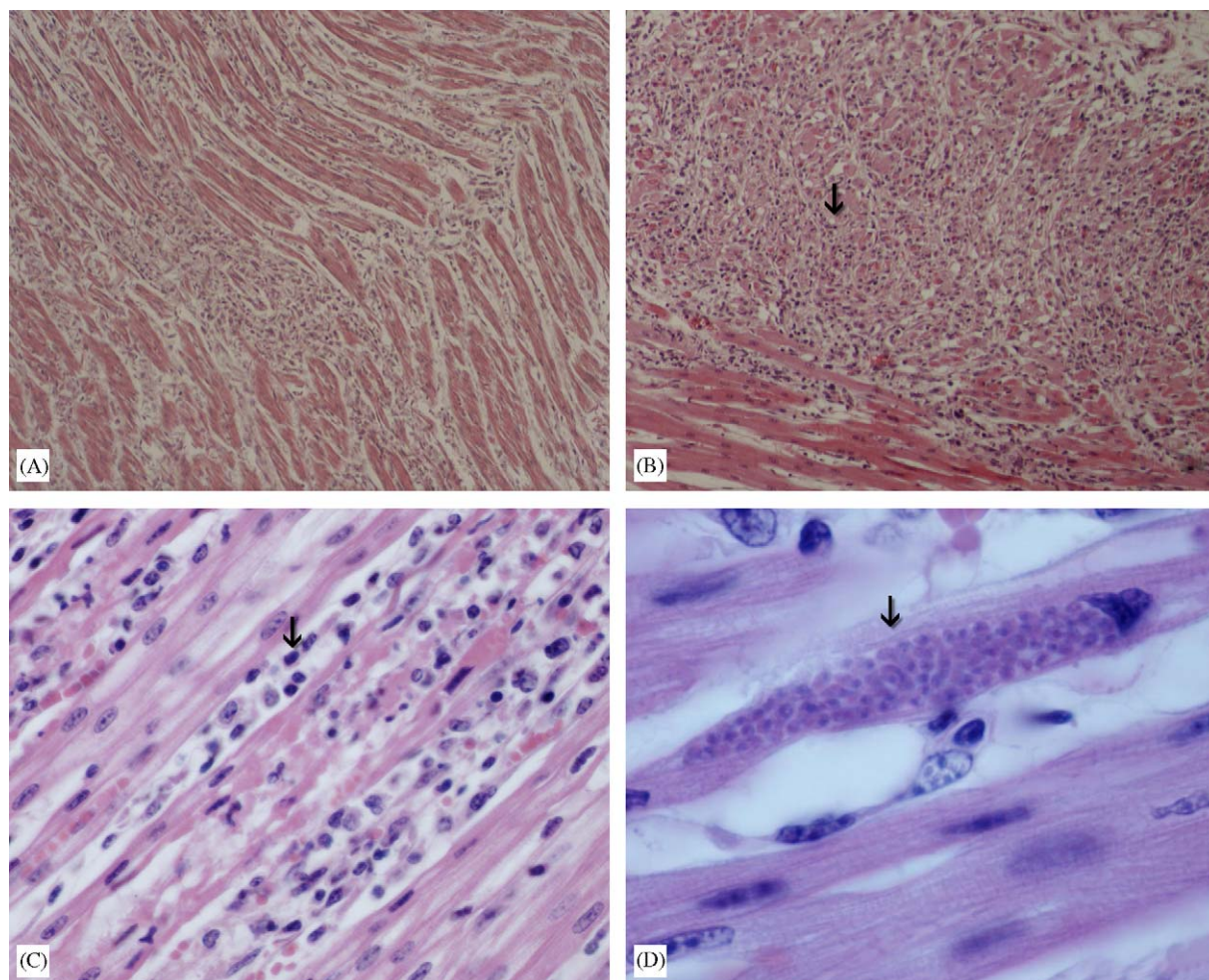
Fig. 6. A–D. Myocarditis associated with *N. caninum* infection in naturally infected bovine fetuses. (A) Infiltration of mononuclear cells in the myocardium of a fetus. HE. × 150. (B) Severe epicardial myocarditis with necrosis (arrow) in a stillborn fetus. HE. × 150. (C) Focal necrosis and infiltration of mononuclear cells in the myocardium of a fetus. HE. × 300. (D) A large group of intracellular tachyzoites (arrow). It is rare to find well preserved tachyzoites in bovine fetuses. HE. × 600.

the brain, being characterized by focal gliosis and peri-vascular infiltrations of mononuclear cells. Tissue cysts, rather than tachyzoites, were found in these calves and extraneural lesions were not reported, except in the skeletal muscles of two of them (Peters *et al.*, 2001).

Myocardial lesions, which may be severe but are often masked by autolysis, typically consist of focal infiltration by mononuclear cells with minimal necrosis. In a grossly affected, stillborn calf, extensive myocarditis (Fig. 6B,C) was observed, together with necrosis of cardiomyocytes and numerous *N. caninum* tachyzoites throughout the heart. Only a few protozoa were found in the brain (Dubey *et al.*, 1990b). Hepatic lesions consisted of periportal infiltrations of mononuclear cells as well as foci of necrosis of variable size and number in the parenchyma, and sometimes associated intrasinusoidal fibrin thrombi (Barr *et al.*, 1990; Wouda *et al.*, 1997). Periportal hepatitis and multifocal hepatocellular necrosis (but not muscular or neural lesions) were

more severe in epidemic than sporadic abortions (Wouda *et al.*, 1997). In an atypical case of neosporosis (Dubey *et al.*, 1992a) a 4-week-old calf that was clinically normal at birth started to develop symptoms when aged 2 weeks. At necropsy a severe non-suppurative encephalitis was found, characterized by large areas of necrosis, mononuclear cell perivascular cuffs and significant groups of tachyzoites. There was also myositis, nephritis and pneumonitis, with *N. caninum* tachyzoites associated with the muscle lesion.

The number of *N. caninum* organisms found in bovine fetal tissues is typically low, even in well-preserved dead fetuses. The difficulty in finding tachyzoites in routine histological sections may be due in part to the pathogenesis of placental lesions discussed above, with either maternal immune "expulsion" or prostaglandin-induced luteolysis causing premature uterine contractions and fetal expulsion before the parasite has time to multiply to any great extent in the fetus.
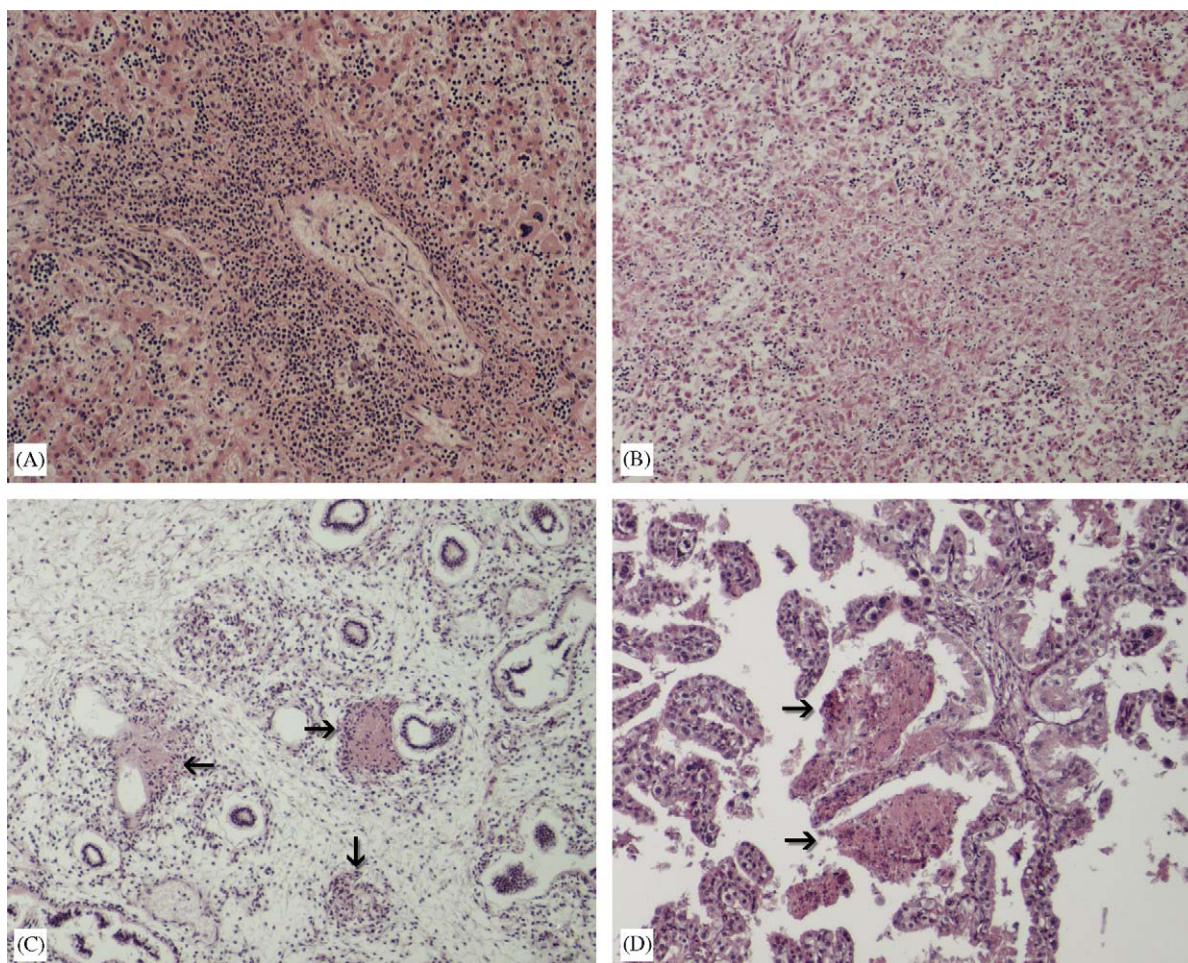
Fig. 7. A–D. Necrosis and inflammatory lesions in bovine fetuses. HE. (A) Periportal hepatitis. HE. × 150. (B) Focal necrosis and hepatitis in a 5-month gestational age fetus. HE. × 150. (C) Focal necrosis (arrows) in the lung of a 3-month gestational age fetus. HE. × 150. (D) Focal necrosis (arrows) in the placenta of the same fetus as in Fig. 6C. HE. × 150.

Histopathological lesions characteristic of neosporosis may be found in several organs, of which the fetal brain is most consistently affected. It is clear from experimental studies (Table 2) that the brain is parasitized early in the cycle. Tachyzoites multiplying in and around small blood vessels in the brain initiate encephalitis (Barr *et al.*, 1991a; Dubey *et al.*, 1992b) and it is likely that in a proportion of these cases encephalitis kills the fetus. It is of interest that viable *N. caninum* has rarely been recovered in cell cultures seeded with tissues from fetuses histologically proven to be infected with *N. caninum* (Conrad *et al.*, 1993, Table 1). In diagnostic terms *N. caninum* is rarely found in tissues without lesions (Boger and Hattel, 2003) and immunohistochemistry is more reliable than conventional HE-stained sections for demonstrating the parasite.

Conclusions

Within a very few years of *N. caninum* being first recognized (Bjerkås *et al.*, 1984) it became apparent that this protozoan parasite is a significant primary cause of bovine abortion throughout the world. The parasite is passed from mother to daughter with ease and in only a minority of cases does it cause fetal death; when this occurs, however, such losses are of considerable economic significance to farmers. While endogenous transplacental transmission is the principal mechanism of parasite survival, the evolution of *N. caninum* has also resulted in oocysts remaining an essential component in the cycle of events. Unravelling the circumstances that govern their production by canids remains an urgent and important task for scientists and success will allow the development of more informed measures of control of bovine neosporosis. Control also requires a thorough understanding of its pathogenesis. As touched on above, the question of how *N. caninum* kills the fetus exposes the complex and finely balanced biological processes that have evolved to permit bovine and other mammalian pregnancies to occur. Defining these immunological mechanisms will not only shed light on potential methods of control of

bovine neosporosis but will enrich our understanding of the continuity of mammalian and protozoal survival.

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